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REGULAR ARTICLE

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE LEPR CANDIDATE GENE WITH CARCASS TRAITS OF PIGS

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ABSTRACT

Leptin and leptin receptor genetic variants are associated with obese phenotypes in humans and mice and are expected to influence fat deposition in pigs. This study aimed to investigate the associations of *LEPR* polymorphism with carcass traits (half carcass weight, lean meat percentage, back-fat thickness, MLT area - m*usculus longisimus thoracis*) and evaluation of genotypic values, additive values and dominance deviations. To identify the genotypes of *LEPR* candidate genes, we used biological material obtained from sows (55) and boars (51) of hybrid combination Large White x Landrace after reaching the slaughter weight. We identified three genotypes using restriction endonuclease *Hpa*II in a group of 106 pigs. The AA genotype was the dominant one (42.45%), AB heterozygotes constituted 39.62%, while the BB genotype was the lowest (17.93%). Analyzing the half carcass weight the highest value detected was the highest in the dominant AA homozygotes together with the highest genotypic value (G_{AA} = 0.3649). The pork genotype AA presented the highest back-fat thickness, A high correlation between the additive genetic effect of the A allele and back-fat thickness (0.8183) has been observed while the effect of the allelic dominance was relatively low (0.1907). Based on our results we may conclude that there is an

inverse and antagonistic relationship between the quality of the half carcass weight together with the back-fat thickness and the lean meat marker.

Keywords: pig, *LEPR* (*Hpa*II), carcass trait, genotypic value

INTRODUCTION

In process of pig interbreeding, as well as of other farming animals, the aim of breeders is to achieve the best possible productive results and traits derived from several molecular-genetic methods.

Leptin is a protein hormone produced primarily by white adipose tissue and involved in regulation of feed intake, energy expenditure, growth and body composition, as well as immune system functions and several aspects of reproduction **(Houseknecht *et al.*, 1998)**. Polymorphisms of this gene in pigs was studied by **Stratil *et al.* (1997)**, **Kennes *et al.* (2001)**, **Szydlowski *et al.* (2004)**, **Stachowiak *et al.* (2007)** and by **Lagonigro *et al.* (2003)**, **Moravčíková *et al.* (2012a)** in cattle.

Genetic variants of leptin and the leptin receptor are associated with obese phenotypes in humans and mice and are expected to influence the fat deposition in pigs **(Friedman and Halaas, 1998).** Possible genotypic association between the leptin gene and genotypes for milk production was observed in cattle **(Moravčíková *et al.*, 2012b)**.

Ernst *et al.* (1997) localized the leptin receptor gene (*LEPR*) on chromosome 6 (SSC6) and is related to the control of feed intake and the regulation of energy balance in mammals since it modulates the leptin effect. Polymorphisms of *LEPR* gene in pigs was studied by **Vincent *et al.* (1997)**(*Hinf*I)**, Stratil *et al.* (1998)** (*Hpa*II *a Rsa*I) and by **Liefers *et al.* (2004)**(*Taq*I), **Trakovická *et al.* (2011)**(*Bse*GI) in cattle.

The *LEPR* gene encodes leptin receptor, a member of the class I cytokine receptor family **(Tartaglia *et al.* 1995)**. Due to the fact that the satiety and fat deposition effects of leptin **(Houseknecht and Portocarrero, 1998; Houseknecht and Spurlock, 2003; Halaas and Friedman, 1997)** are mediated through the leptin receptor, the leptin receptor gene (*LEPR*) is also considered a candidate for traits related to growth and body composition **(Li *et al.*, 2010)**. In pigs, some polymorphisms within *LEPR* gene are related with backfat deposition, daily gain **(Vincent *et al.*, 1997, Muñoz *et al.*, 2008)** and IMF content **(Stratil *et al.*, 1998)**.

The objectives of the present study were to analyse of the *LEPR* (*Hpa*II) polymorphism and to estimate its genotypic and allelic frequencies in hybrid combination of Large White x Landrace. This study also aimed to investigate the associations of LEPR polymorphism with carcass traits (half carcass weight, lean meat percentage, back-fat thickness, MLT area -m*usculus longisimus thoracis*) and evaluation of genotypic values.

MATERIAL AND METHODS

To identify the genotypes of *LEPR* candidate genes, we used biological material obtained from sows (55) and boars (51) of hybrid combination Large White x Landrace after reaching the slaughter weight (90-110 kg).

Blood samples were collected in tubes with anticoagulant solution. Samples were until the start of the analysis frozen.

All individuals were reared in the same conditions and fed with standard feed mixtures.

DNA was isolated from blood samples of animals according to **Miller *et al.* (1988)**. PCR RFLP method was adapted to the conditions of our laboratory **(Kováčik, *et al.* 2011)** and for amplification we used following oligonucleotides primers FOR 5´ GGA AGG CAT TTG TTT CAG CAG TAA 3´ and REV 5´ CAA GTC CTC TTT CAT CCA GCA CTG 3´ **(Stratil et al. 1998)**. PCR reaction mix (25 µl) contained: MgCl2 1.5 mM, dNTP 200 µM, primers 0.5 µM a 0.5 U Taq DNA polymerase, DNA 1 µl. After amplification, the PCR product was digested with *Hpa*II.

The observed carcass traits (half carcass weight, lean meat percentage, back-fat thickness and MLT area - *musculus longisimus thoracis*) were measured by standard methodology **(STN 466164)**.

For the calculation of genotypic and additive value, we used the program GENETICS3.

RESULTS AND DISCUSSION

We identified three genotypes using restriction endonuclease *Hpa*II in a group of 106 pigs. The AA genotype was the dominant one (42.45%), AB heterozygotes constituted 39.62%, while the BB genotype was the lowest (17.93%). We observed a higher frequency of the A allele (0.6227) compared with the B allele (0.3773) in the test group.

Amills *et al.* (2008) studied *LEPR* (*Hpa*II) genotypes and their association with a differential level of plasma *LEP* concentration as well as with phenotypic variation at several production traits in a Landrace population and confirmed the high frequency of BB genotype (AA: 0.034, AB: 0.307 and BB: 0.659).

Analyzing the half carcass weight the highest value detected was the highest in the dominant AA homozygotes together with the highest genotypic value (G_{AA} = 0.3649), while the additive genetic value in relation to the development of this characteristics was 0.1914 and the effect of the allelic dominance A was 0.1708 (Table 1).

The pork genotype AA presented the highest back-fat thickness, a result which was located at the lowest limit of significance in relation to the genotypes AB and BB. A high correlation between the additive genetic effect of the A allele and back-fat thickness (0.8183) has been observed while the effect of the allelic dominance was relatively low (0.1907), which means that the main part of the genotypic value was constituted by the additive effect (Table 2).

Óvilo *et al.* (2002) showed that *LEPR* genotypes are associated with back-fat thickness and intramuscular fat content (between two genotypes for BFT: BB – AB = 2.96 ± 0.94 mm → $P < 0.0018$). This association seems to be high since the difference between the two genotypes on back-fat thickness corresponds to 0.37 phenotypic standard deviations.

Óvilo *et al.* (2005) tested Iberian x Landrace experimental population to *LEPR* coding regions and examined their effects on fatness and body composition traits. Authors found significant associations with back-fat at first rib (additive effect = 0.331; dominance effect = - 0.086), weight of bacon (additive effect = 0.299; dominance effect = - 0.095) and weight of ribs with sternum (additive effect = 0.428; dominance effect = 0.299).

No significant differences were found when examining the lean meat characteristic, however the values were in favor of the BB individuals (55.68), which is confirmed with the estimated genotypic values (G_{AA} = –0.3610, G_{AB} = 0.0589, G_{BB} = 0.7889). Based on the data we may conclude that the studied group exhibited the highest genotypic value for the BB genotype in relation to the lean meat characteristics, with an additive effect of 0.6687 and highly superating the effect of allelic dominance (0.6687 > 0.1202; Table 3).

Studying the MLT area (*musculus longisimus thoracis*) no significant differences were detected, however it may be suggested that the AA genotype has an impact on this parameter (Table 4).

The results of many studies **(Costa *et al.*, 2004; Zhang *et al.*, 2007; Zhao *et al.*, 2010)** confirm that by modifying nutrition one can influence the expression level of genes, and

enables obtaining much higher limits of traits controlled by these genes (carcass traits) compared to animals receiving standard feed. To make this mechanism of action possible, it is necessary to identify genetic control of traits of interest and to understand metabolic pathways **(Tyra and Ropka-Molik, 2011)**.

Li *et al.* (2010) chose *FABP3* and *LEPR* as candidate genes for meat texture traits and fatness parameters in Korean native pigs. The results obtained by these authors clearly indicate that mutation *LEPR* c.2856C > T identified by endonuclease *Ava*II is significant associate with several meat quality traits including intramuscular fat.

Guay *et al.* (2001) indicated leptin and leptin receptor may play a role during early stages of development of the pig embryo-fetus, and this role could be modulated according to the breed and parity of the sows.

Table 1 *LEPR* genotypic values for the half carcass weight

Genotypes LEPR	BB	AB	AA
Measured values (kg)	40.87	40.38	41.09
Genotypes	Genotypic values, G	Additive values, A	Dominance deviations, D
AA	**0.3649**	**0.1914**	**0.1708**
AB	-0.3450	-0.0631	-0.2819
BB	0.1449	-0.3203	0.4653
deltaq	VarT = 0.1105	VarA = 0.0310	VarD = 0.0794

Table 2 *LEPR* genotypic values for the back-fat thickness

Genotypes LEPR	BB	AB	AA
Measured values (mm)	17.21	17.46	19.05
Genotypes	Genotypic values, G	Additive values, A	Dominance deviations, D
AA	**1.0090**	**0.8183**	**0.1907**
AB	-0.5809	-0.2661	-0.3148
BB	-0.8309	-1.3505	0.5159
deltaq	VarT = 0.6516	VarA = 0.5525	VarD = 0.0991

Table 3 *LEPR* genotypic values for the lean meat

Genotypes LEPR	BB	AB	AA
Measured values (%)	55.68	54.95	54.53
Genotypes	Genotypic values, G	Additive values, A	Dominance deviations, D
AA	-0.3610	-0.4051	0.0441
AB	0.0589	0.1317	-0.0728
BB	**0.7889**	**0.6687**	**0.1202**
deltaq	VarT = 0.1407	VarA = 0.1354	VarD = 0.0053

Table 4 *LEPR* genotypic values for the MLT (*musculus longisimus thoracis*)

Genotypes LEPR	BB	AB	AA
Measured values (cm^2)	43.28	43.68	43.84
Genotypes	Genotypic values, G	Additive values, A	Dominance deviations, D
AA	**0.1549**	**0.1890**	**0.0341**
AB	-0.0050	-0.0614	-0.0563
BB	-0.4050	-0.3120	-0.0930
deltaq	VarT = 0.0326	VarA = 0.0294	VarD = 0.0031

CONCLUSION

Based on our results we may conclude that there is an inverse and antagonistic relationship between the quality of the half carcass weight together with the back-fat thickness and the lean meat marker. It is necessary to observe and evaluate the heritability and genotypic correlations between the studied characteristics.

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